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Oral bait preferences and feasibility of oral rabies vaccination in Bangladeshi dogs

Jesse Bonwitt [a,b,*], Sarah Bonaparte [a,c], Jesse Blanton [d], Andrew D. Gibson [e,f], Mahbub Hoque [g], Erin Kennedy [h], Kamrul Islam [i], Umme Ruman Siddiqi [i], Ryan M. Wallace [a], Shakif Azam [j]

[a] Poxvirus and Rabies Branch, Division of High Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, GA, USA
[b] Department of Anthropology, Durham University, Durham, United Kingdom
[c] ORISE Fellow, Centers for Disease Control and Prevention, Atlanta, GA, USA
[d] Divison of Global Migration and Quarantine, Centers for Disease Control and Prevention, Atlanta, GA, USA
[e] Mission Rabies, Cranborne, UK
[f] The Roslin Institute and The Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush Veterinary Centre, Roslin, Midlothian, United Kingdom
[g] TEPHINET, Centers for Disease Control and Prevention, Dhaka, Bangladesh
[h] Global Immunisation Division, Centers for Disease Control and Prevention, Atlanta, GA, USA
[i] Zoonotic Disease Control Programme, Communicable Disease Control Unit, Directorate General of Health Services, Dhaka, Bangladesh
[j] Department of Livestock Services, Ministry of Fisheries and Livestock, Dhaka, Bangladesh

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ABSTRACT

Oral rabies vaccination (ORV) can increase rabies vaccination coverage among dogs that are inaccessible to parenteral vaccination (i.e., inaccessible dogs). Because bait uptake can differ according to the bait attractant used and dog characteristics, we evaluated proportion of bait uptake and time to bait uptake using three bait formulations. We looked for associations between bait uptake and dog characteristics (temperament, age, and body condition) and assessed the efficiency of using these bait formulations, as measured by number of dogs vaccinated per hour.

A total of 356 baits were offered to free roaming dogs in urban and *peri*-urban districts of Bangladesh. Fish baits were ignored by 86% (n = 122; 95% CI: 79–91%) of dogs, whereas 60% (n = 45; 95% CI: 49–70%) consumed egg baits and 89% (n = 124; 95% CI: 83–93%) consumed intestine baits. Among the consumed baits, dogs fully consumed 56% (n = 10; 95% CI: 34–75%) of fish baits, 84% (n = 38; 95% CI: 71–92%) of egg baits, and 98% (n = 122; 95% CI: 94–100%) of intestine baits. Among inaccessible dogs, no associations were found between bait uptake and dog characteristics in either bivariate or multivariate analyses. Bait consumption averaged 2 dogs per hour for fish baits, 10 dogs per hour for egg baits, and 18 dogs per hour for intestine baits.

The absence of association between bait type preference and individual dog characteristics simplifies the process of choosing attractants for oral rabies vaccines. While intestine attractants achieved highest uptake, egg baits may prove a suitable compromise when considering biological and operational constraints. The efficiency of ORV was demonstrated when compared to parenteral vaccination of free-roaming dogs previously described.

1. Introduction

Following the successful use of oral rabies vaccines in the elimination of sylvatic fox rabies in western Europe [13], and fox and coyote rabies virus variants from the southern United States [12], oral rabies vaccination (ORV) has been proposed as a complementary method for the elimination of canine rabies [18]. ORV is particularly well suited for vaccinating dogs that are inaccessible to parenteral vaccination (e.g., free-roaming owned dogs and feral dogs), or for which parenteral vaccination using capture-vaccinate-release (CVR) requires disproportionate resources to achieve the 70% vaccination coverage necessary to eliminate canine rabies.

Recent advances in ORV efficacy are promising, and several field studies have shown that ORV in combination with CVR and mobile central point (MCP) vaccination can increase vaccination coverage

* Corresponding author at: Poxvirus and Rabies Branch, DHCPP, CDC, 1600 Clifton Road NE, Atlanta, GA 30333, USA.

E-mail address: jbonwitt@cdc.gov (J. Bonwitt).

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in communities with free-roaming dog populations [8,15]. Despite these encouraging findings, ORV for dogs has not been widely adopted in mass vaccination campaigns on a variety of grounds, including perceived uncertainty regarding vaccine safety (for accidental human exposure), vaccine efficacy and stability, bait uptake, and overall cost-benefit [14,18,4].

ORV used in dogs consists of a liquid vaccine formulation enclosed within a sachet, which is coated with a bait attractant. Bait uptake is dependent upon the attractiveness, palatability, and design of the bait, among other factors, and considerable differences in preference have been documented among species and even within the same species [4,8,5]. Tailoring such gastronomic and physical particularities to target animals is critical to ensuring that they accept the bait and that the vaccine is appropriately delivered into the oral cavity. Attempts to identify associations between dog characteristics, such as age, sex, and nutritional status, and bait uptake have shown mixed results [3,11]. Significantly, establishing the effect of dog temperament on bait uptake will be key to ensuring that inaccessible dogs—the primary intended target for ORV—consume the bait.

In 2018, Bangladesh launched a mass canine rabies vaccination campaign using parenteral vaccines delivered primarily through a CVR methodology. In line with recommendations by the World Health Organization that ORV be piloted before widescale application [18], we performed an initial assessment of ORV feasibility using bait constructs without active vaccine in pilot areas of Bangladesh as part of this larger vaccination campaign. As there are currently no licensed oral rabies vaccines in Bangladesh, this is an important step prior to further evaluation of candidate oral rabies vaccines. The objective of our study was to compare bait uptake and time to bait uptake using three bait formulations. Bait uptake was compared by dog temperament, age, and body condition.

2. Methods

2.1. Study context

Bait attractant evaluations took place over four days between 30 July and 25 September 2018, during a national canine rabies vaccination campaign in the Dhaka and Chittagong Divisions of Bangladesh. These divisions were chosen based on the presence of human rabies surveillance [10] and priorities set by the national canine rabies control program. In each of the two divisions, we selected one urban (Chittagong Kotwali [population density: 41,658 per km^2] and Narayanganj City Cooperation [population density: 47,000 per km^2]) and one *peri*-urban (Meghna [population density: 1129 per km^2] and Sreepur sub-district [population density: 1064 per km^2]) site (Fig. 1) [1]. Within these locations, ORV evaluation zones were chosen to represent areas with similar estimated dog density (51 dogs per km^2), which were inferred from a human to free-roaming dog population estimate used for vaccination program planning (300:1 for urban areas, 150:1 for *peri*-urban) and the most recent human population census.

2.2. Bait attractant

We evaluated three bait attractants (Table 1, Fig. 2). Because oral rabies vaccines are not licensed in Bangladesh, we used placebo oral rabies vaccines only; no vaccine or vaccine sachets were used in this evaluation. Fishmeal baits consisted of a block of vegetable fatty acids and fishmeal. Egg baits were of the same material used in previous studies [3,11]. Intestine baits were assembled from locally purchased cow intestine. Intestines were boiled for 5 minutes and then cut into 8–10 centimeter segments to approximate the size required to hold a vaccine sachet.

2.3. Bait distribution

The national vaccination campaign utilized a CVR approach [16], which operated in each of the four ORV evaluation zones for 3–5 days, depending on the number of dogs that vaccinators encountered. ORV evaluations took place within 2–3 days of completing CVR vaccination. An ORV evaluator started the assessment in the centroid of each selected zone and used a simple random direction generator to complete a transect. Two transects were each conducted in Narayanganj City Cooperation and Sreepur sub-district, and one transect was each conducted in Chittagong Kotwali and Meghna sub-district. ORV evaluators were given 60 baits of a single type and instructed to continue the evaluation until they had offered baits to at least 30 dogs. Six evaluators participated in the study, all whom had similar multi-year experience interacting with free-roaming dogs through national vaccination campaign activities. Each ORV evaluator was randomly assigned a bait type and was reassigned a different attractant when moving to the next evaluation zone to limit any potential differences in baiting capabilities between evaluators. Baits were stored in a walk-in refrigerator until the morning of use, at which time they were transferred to a hard-shell hand-held cooler that contained one block of ice. All baits were thawed when distributed in the field. ORV evaluators (one vaccinator and one data collector) approached free-roaming dogs indirectly on foot and watched them from a distance as previously described [8]. Baits were offered to free roaming dogs that could be approached within approximately 3 meters to enable the vaccinator to gently throw the bait in front of the dog. Dogs in crowded or unsafe locations (e.g. in a busy street) were not selected. When multiple dogs were in one location, vaccinators attempted to distribute baits to individual dogs in an effort to separate dogs. Dogs were only offered a bait once, and vaccinators ceased offerings baits if dogs became aggressive. Dogs that consumed a bait were marked with a non-toxic water-based paint using spray bottles to avoid offering the same dog more than one bait. Dogs were given up to 5 min to consume the bait, after which time the bait was retrieved or abandoned if the dog was too aggressive to approach. Retrieved baits that had not been damaged were re-used.

2.4. Bait uptake assessment

Prior to starting field work, a 2-day session was held on evaluation methods, data collection, and field exercises. ORV evaluators were members of the CVR vaccination teams, and all had multiple years of experience capturing and vaccinating dogs as part of the national rabies vaccination campaign. A mobile phone application was used to record ORV evaluation data [7]. The path transected by the ORV evaluator was captured, as was a GPS point for each dog encountered. Information pertaining to the site included site name, date, oral bait attractant, start time, and stop time. For every dog that was offered a bait, an observer recorded the bait contact (“showed interest” or “ignored”), bait consumption (yes or no), proportion of the bait consumed (“little”, “mostly”, or “complete”), and bait consumption time. Bait consumption time was recorded as the time elapsed between a dog being offered a bait and consuming it or losing interest, measured in time intervals (<30, 30–60, 61–120, or >120 seconds). Bait consumption time was only recorded for dogs that consumed at least part of the bait.

ORV evaluators were trained to record dog characteristics including age (juvenile, adult, or unknown), body condition (skinny, ideal, or overweight), and temperament (timid, aggressive, or friendly). The age of the dog was estimated by the evaluator;

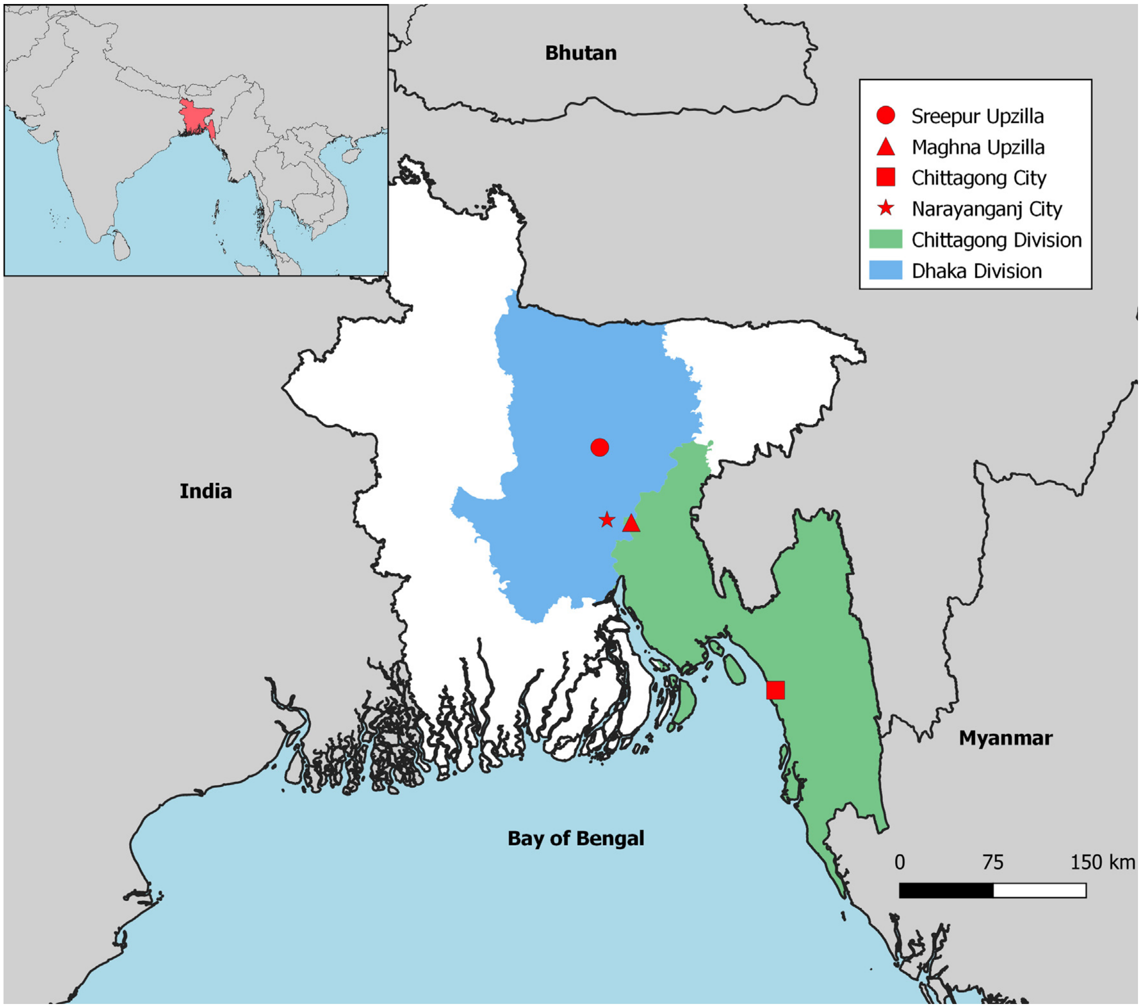

Fig. 1. Study sites.

Table 1
Characteristics of the three bait attractants used.

Bait attractant	Size	Weight	Formulation	Sachet
Fishmeal	8.5 × 4.0 × 1.2 cm	43 g	Fishmeal, vegetable fatty acids	None
Egg	8.5 × 4.0 × 1.2 cm	43 g	Whole egg powder (chicken), gelatin	None
Cow intestine	10.0 × 3.0 × 3.0 cm	40–60 g	No additives	None

adult dogs were classified as ≥1-year-old and juvenile dogs were classified as <1-year-old. The temperament of the dog was determined at the discretion of the ORV evaluators.

2.5. Statistical analyses

Data were uploaded daily to a cloud-based server and downloaded by evaluation supervisors as an Excel document for review and analysis. Analyses were performed using SAS 9.4 (SAS Institute Incorporated, Cary, NC, USA) and Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA).

Bait preferences were assessed independently across bait attractants for each of the categorical variables collected. Confidence intervals were calculated around the mean values. In the multivariable model, free-roaming dogs were stratified by temperament as either "accessible," (if reported as friendly), or "inaccessible," (if reported as timid, aggressive, or of unknown temperament). Bait attractants were stratified by their effectiveness as either "high-uptake" (egg and intestine baits), or "low-uptake," (fishmeal baits). To assess characteristics of the inaccessible dogs who consume baits (i.e., the target dog population for ORV), bivariate and multivariable log-binomial regressions were conducted. Independent variables of interest included type of bait attractants, age, body condition, and site type (urban or *peri*-urban), while the binary dependent variable of interest was bait uptake. Low-uptake bait attractants (i.e., fishmeal) were excluded from the analysis because they were considered ineligible for use in future ORV campaigns. Site type was included as an independent variable to account for the possible effects of clustering of data within urban and *peri*-urban sites. Rate ratios (RRs) with corresponding 95% confidence intervals are reported using a p-value cutoff of <0.05.

2.6. Ethical approval

The evaluation was deemed non-research by the CDC institutional review board and was reviewed by the Bangladesh Ministry

Fig. 2. Bait attractants used in study (left to right: raw cow intestine, egg, fishmeal).

of Health and Family Welfare. Field activities related to animal observations were covered under CDC's Institutional Animal Care and Use Committee protocol (#2757DOTMULX-A3).

3. Results

A total of 356 baits were offered to free roaming dogs, including 102 (29%) in Narayanganj City, 63 (18%) in Chittagong City, 125 (35%) in Meghna Upzilla, and 66 (19%) in Sreepur Upzilla (see supplementary materials for maps and GPS stamps). Bait attractants consisted of fish (n = 142, 40%), egg (n = 75, 21%), and intestine (n = 139, 39%).

Fish baits were ignored by 86% (n = 122; 95% CI: 79–91%) of dogs, whereas 60% (n = 45; 95% CI: 49–70%) consumed the egg baits and 89% (n = 124; 95% CI: 83–93%) consumed the intestine baits (Fig. 3). Among the consumed baits, 56% (n = 10; 95% CI: 34–75%) of fish baits, 84% (n = 38; 95% CI: 71–92%) of egg baits, and 98% (n = 122; 95% CI: 94–100%) of intestine baits were fully consumed.

In general, the relative proportion of uptake by bait attractant stayed similar across age and body condition score. Baits were offered to 44 friendly dogs, 262 timid dogs, 46 aggressive dogs, and 4 dogs of unknown temperament (Table 2). A higher proportion of friendly dogs (n = 42, 95%) accepted baits than timid (n = 131, 50%) or aggressive (n = 13, 28%) dogs. All friendly dogs accepted egg and fish baits, whereas timid and aggressive dogs showed a heightened preference for intestine baits (Table 2).

In the bivariate analysis, the probability of inaccessible dogs consuming intestine baits was 1.8 times higher than the probability of consumption among those offered egg baits (RR = 1.8; 95% CI: 1.4–2.4). The probability remained significant when controlling for body condition, age, and vaccination site (RR = 1.8; 95% CI: 1.4–2.4) (Table 3). No associations were found between bait uptake and dog characteristics among inaccessible dogs in either the bivariate or multivariate analysis.

The proportion of dogs consuming baits ≤61 seconds was 41% (95% CI: 21.6–64.0%) for fish baits, 67% (95% CI: 52.1–78.6%) for egg baits, and 96% (95% CI: 90.8–98.3%) for intestine baits. GPS and time-stamp data showed that consumption of ORV baits occurred at an average of 2 dogs per hour for fish baits, 10 dogs per hour for egg baits, and 18 dogs per hour for intestine baits. An average of 12 dogs were offered baits per hour in urban sites and 9 dogs per hour in *peri*-urban sites. However, bait uptake did not vary significantly by site type in either the bivariate or multivariate analysis (Table 3).

4. Discussion

An estimated 2000 people die from rabies virus infection and more than 40,000 people receive post-exposure prophylaxis annually in Bangladesh [10]. Although current dog vaccination has failed to reach the 70% vaccination coverage necessary to attain herd immunity [10,2], a recent decrease in human rabies incidence attributed to an increase in mass dog vaccination lends hope that human and canine rabies can eventually be controlled through sustained vaccination [6].

We found that dogs tend to prefer intestine baits over egg and fish, and preferred egg over fish baits (Fig. 3). Our results are similar to those found in free-roaming dogs in Najavo Nation, United States, where a lower bait acceptance in fish (81.1%) and egg baits (77.4%) compared to intestine baits (91.9%) was attributed to lack of familiarity with their taste, smell, and texture [3]. Although acceptance of fish bait was higher than egg baits in the Navajo

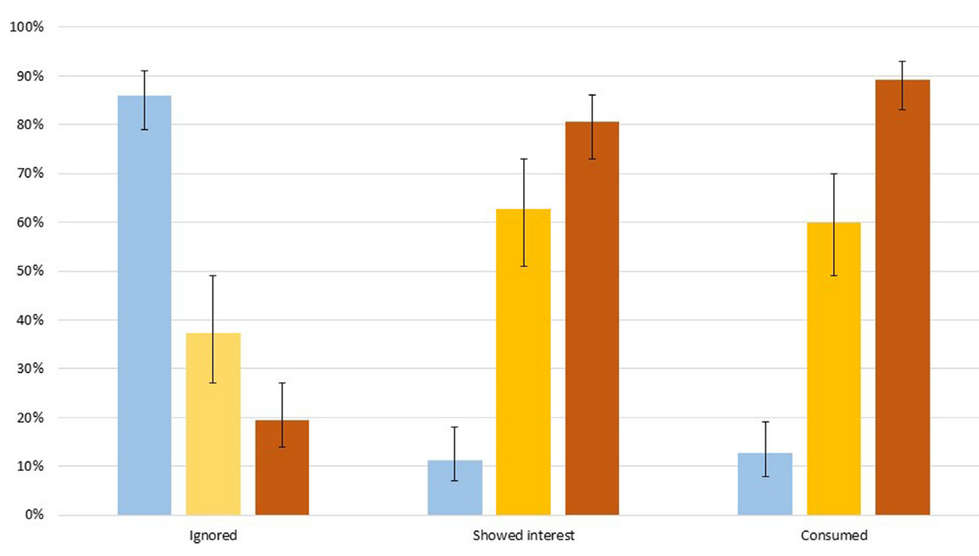

Fig. 3. Response to baits (mean proportion and 95% CI) by bait attractant, N = 356. Bait response is non-exclusive, i.e. dogs can show interest in a bait and consume it.

Table 2
Bait uptake (fish, egg, or intestine) by dog characteristics (body conditions score and age), and dog temperament (friendly, timid, or aggressive), N = 356.

	N	Fish (% n/N)	Egg (% n/N)	Intestine (% n/N)
	Body condition score			
Average	275	12% (15/124)	45% (19/42)	92% (100/109)
Underweight	50	10% (1/10)	78% (18/23)	76% (13/17)
Overweight	31	25% (2/8)	80% (8/10)	85% (11/13)
	Age			
Adult	241	11% (12/108)	54% (30/56)	91% (70/77)
Juvenile	82	29% (6/21)	88% (14/16)	89% (40/45)
Unknown	33	0% (0/13)	33% (1/3)	82% (14/17)
	Temperament			
Friendly	44	100% (5/5)	100% (16/16)	91% (21/23)
Timid	262	10% (11/105)	52% (28/54)	89% (92/103)
Aggressive	46	7% (2/29)	0% (0/4)	85% (11/13)
Unknown	4	0% (0/3)	100% (1/1)	(0)

Table 3
Rate ratios of bait uptake (egg or intestine) among inaccessible dogs (defined as dogs recorded as aggressive, timid, or of unknown temperament), N = 175. Bolded font with * indicates a significant association ($p < 0.05$).

		Bivariate model		Multivariate model	
	N	Rate Ratio	95% CI	Rate Ratio	95% CI
Bait attractant					
Egg	59	Ref		ref	
Intestine	116	**1.8***	1.4–2.4	**1.8***	1.4–2.4
Body condition score					
Average	135	ref		ref	
Underweight	28	0.9	0.7–1.2	0.9	0.8–1.1
Overweight	12	1.0	0.7–1.4	1.0	0.8–1.4
Age					
Adult	113	ref		ref	
Juvenile	43	**1.2***	1.1–1.4	1.0	0.9–1.3
Unknown	19	0.9	0.7–1.2	1.0	0.7–1.2
Site type					
Urban	97	ref		ref	
Peri-urban	78	1.1	0.9–1.3	0.9	0.8–1.1

study, overall effectiveness (measured by water dye) was highest with intestine (75.4%), followed by egg (68.0%), and fishmeal (54.3%) baits. While this mirrors our findings, acceptance and consumption rates of fishmeal attractant were much lower among dogs in our study. In Bangladesh, offal is traditionally given to free-roaming dogs, and intestine baits are therefore a familiar food among this population, which could explain the greater uptake of intestine baits among free-roaming dogs included in our evaluation. In Thailand, dogs more often refused fishmeal baits than other types of attractants, and intestine baits were entirely consumed [11]. High rates of egg bait uptake were also reported in dogs in Goa, India, with 78% of dogs consuming the bait, and sachet perforation observed in 91% of all dogs consuming the bait [8].

In addition to containing baits that are attractive to dogs, oral rabies vaccines must contain vaccine sachets that can be perforated by teeth during chewing to release the liquid vaccine into the oral cavity [4]. Although we did not evaluate blister perforation, vaccine blisters in egg baits were more frequently perforated than intestine baits, the latter being significantly more likely to be swallowed without perforation of the blister [3]. Because intestine baits were consumed faster than either fish or egg baits in our evaluation, egg baits might be more likely to effectively increase vaccination coverage despite lower bait uptake compared to intestine. Faced with the diversity of preferences for bait attractant among free-roaming dogs across the world [3,8,11,5], ORV manufacturers are confronted with the challenge of developing a universal attractant. Because intestine bait must be manufactured locally, they remain an unrealistic candidate for mass-production and distribution, whereas egg baits can be mass-produced. Although locally made bait has the advantage of being more acceptable to local dog population, egg would seem to be a good compromise, at least among individuals in our study population.

We did not observe any significant difference in bait uptake by dog age or body condition (Table 3), in keeping with previous studies [3]. The absence of differences among dog characteristics simplifies the process of selecting attractant and distribution logistics.

Oral rabies vaccines should ideally be used in instances where parenteral vaccination is neither possible nor cost-effective [4]. Parenteral vaccination remains the method of choice when dogs can be easily, safely, and rapidly caught and handled, as parenteral vaccines are more thermostable than oral vaccines and induce a protective immune response with a higher degree of certainty [18]. Although bait uptake was relatively high among inaccessible dogs for egg (48%) and intestine (89%) baits, they did not match uptake among accessible dogs (95%) (Table 2), highlighting the potential difficulties of vaccinating certain inaccessible dogs.

Previous studies have shown that ORV campaigns might be more effective at vaccinating free-roaming dogs, with the higher cost of oral rabies vaccines compared to parenteral vaccines offset by reduced personnel and logistics costs necessary to conduct CVR [9,17]. In our study, the efficiency of ORV was demonstrated with an estimated 60 dogs vaccinated per vaccinator per day using egg attractants and 108 dogs vaccinated per vaccinator per day using intestine attractants (assuming 6 hours of field work per day). This compared with only 9 dogs vaccinated per vaccinator per day using CVR in India—a relatively high personnel cost per vaccinated dog [9]. In our study, evaluators were able to "vaccinate" 10 dogs per hour using egg baits, which was somewhat less than the 12 dogs vaccinated per hour achieved by Gibson et al. [9] in India. This difference could be due to variation in dog density, or because vaccinators used scooters as a mean of transportation and gained significant experience in distributing oral rabies vaccines over the course of their evaluation (N = 924 baits). Further evaluations are ongoing to compare the efficacy and cost-benefit of ORV and CVR in Bangladesh.

Our evaluation contains several limitations. None of the baits contained a vaccine blister pack, which could have negatively modified bait odor and consistency, and thus overestimate bait uptake (but not bait contact). By not using blister packs, we were also unable to assess whether bait attractants were associated with puncturing blister packs, which is important as vaccine fluid needs to be released within the oral cavity and bait uptake does not necessarily correlate with mounting an immune response [3]. Finally, attractants were randomly assigned to evaluators rather than randomly assigned by dog, which might have caused some bias by evaluator. However, this likely would have been mitigated by randomly assigning baits to evaluators by day.

Oral rabies vaccines hold potential for increasing vaccination coverage, as they can be more efficient in vaccinating inaccessible dogs with less human resources and training needs compared to CVR [9]. Our results suggest that Bangladesh can achieve ORV uptake rates comparable to those reported in India [8], and argue for incorporating ORV into the national vaccination program. Our findings confirm previous results on bait preference studies and specifically show that these results hold true among the target population for ORV (inaccessible dogs). Because bait preferences vary by region [4], preference studies should be evaluated in new settings where socio-cultural and economic differences may influence bait uptake. However, where studies have shown a likely acceptable bait flavor, ORV should not be significantly delayed, and preferences can be evaluated during vaccination activities.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2020.05.047.

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